Effect of premelting on conductivity of DNA-lipid films

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We have measured temperature dependent (between 20 and 80 °C) electrical conductivity and molecular structure (Raman spectroscopy) of DNA-lipid cast film. Our findings show that the conductivity is strongly influenced by premelting effects in the molecular structure starting near physiological temperatures (∼40 °C), prior to the global DNA denaturation.

Most measurements reported in the last decade on the DNA conductivity are conducted at room temperatures and below [1]. If DNA is to become exploitable in microelectronics applications, however, its performance must be reliable at temperatures slightly *above* the room temperature due to the inevitable heating of electronic components. It is well known that DNA molecules, both natural and synthetic, undergo a denaturation process at $T_{dn} = 70 \sim 80$ °C. Above this temperature, the double-stranded molecular conformation is destroyed, and consequently, the electrical conductivity is lost [2, 3]. According to numerous theoretical models [4, 5] even at physiological temperatures (∼40 °C), DNA experiences structural perturbations leading to local denaturations and/or "bubble"-type defect formations. The existence of "bubbles" [6, 7] as well as the temperature induced local perturbations at $T < T_{dn}$, termed "premelting" [8, 9, 10, 11, 12] has been confirmed experimentally, and the biological aspects of these local denaturations were discussed in a number of studies (see for example, [6]). Local deformations should cause breaking of a long-range order in the DNA structure (*i.e.*, interruption of the parallel base-pair (bp) stackings) similar to an order breaking in solid bodies due to the dislocation introduction. But their influence on conductivity has not been properly addressed until now. In this letter, we present the temperature dependent conductivity and structural

evolution monitored through Raman spectroscopy measured on the DNA-lipid cast film between physiological and denaturation temperatures. These DNA-lipid films were previously studied by Okahata *et al.* [2], where disappearance of conductivity above the denaturation temperature was reported. We observed a substantial reduction in the DNA conductivity due to premelting effects starting at temperature as low as 40 °C, lending support to the theoretical inference on the importance of the long range parallel *bp* stacking in DNA for the electrical conduction.

Self-standing DNA-lipid cast films with thickness of about 60 microns were prepared according to the method described elsewhere [13]. Once the self-standing film is mechanically stretched, DNA molecules (natrual DNA, 2000 *bp*'s each) are aligned along film's long axis with an average inter-molecular distance of 41 Å. In previous investigations, Okahata *et al.* have verified the anisotropic conductivity through these films and concluded that the electrons are traveling through the molecules and not via the lipid matrix (conductivity of these molecules was not suppreseed by strong interaction with a solid surface [14]). For our conductivity measurements, a section of a film of about 5x0.5 cm² was placed on a glass plate. The detailed description of measurement apparatus is given in Figure 1. The measurements were performed in a Hewlett-Packard measurement systems in a dark box at temperatures of 30-150 °C and with or without illumination. The leak current through the contacts was less than 1 nA in the measurement range of ± 3 V and for temperatures between 25 and 80 °C, irrespective of illumination. Indentation created by the electrodes in soft insulators, such as resist or teflon, leads only to a reduc-

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tion of the leak current due to an increase in the distance between electrodes. Similar increase is expected in the DNA film, while the total current (I) at 3 V reaches 1 μA and larger. Once heating the film to 150 °C, I again decreases down to $\sim$ 1 nA.
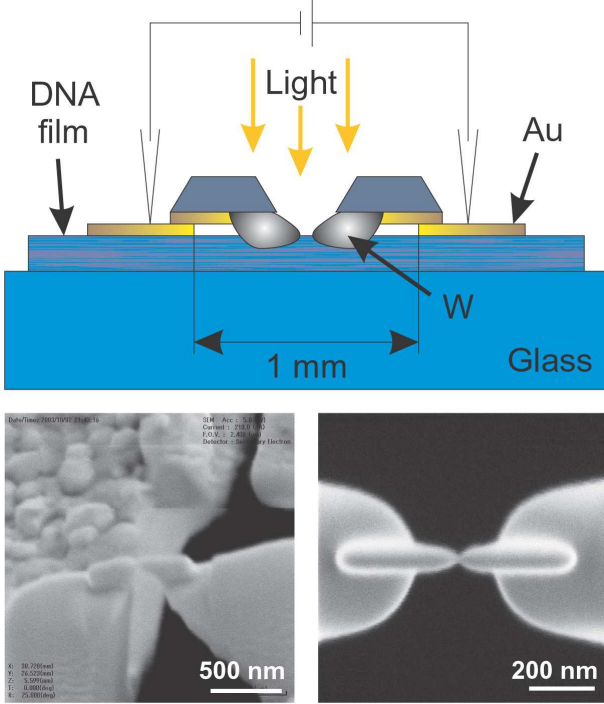

FIG. 1: Conductivity measurement set-up. One micron high tungsten nanoelectrodes with a less than 5 nm separation were prepared by decomposing Tungsten hexacarbonyl by focused ion beam [26] on a silicon chip. The silicon chip was then pressed into the DNA-lipid film surface on which gold contacts had been pre-deposited. Below are SEM (left,side view) and FIB (right,top view) images of the tungsten nanoelectrodes.

With illumination of 3 mW/cm^2 (Halogen photo optic lamp, unfiltered and unpolarized), the overall conductivity and the maximal current ($I_{max} = I(-3V)$) through the DNA-film start to diminish at about 40 °C (Figure 2a). The largest change is observed between 35 and 45 °C (Figure 2b) where I_{max} is reduced by more than 70 %. The stability of the temperature was $\pm$ 0.5 °C during I-V characteristics measurements. When measurements are conducted in dark, the conductivity is 5 times smaller at 35 °C and the temperature dependent reduction of I_{max} is limited to 20 % (not shown). These temperature induced changes in the conductivity are entirely reversible upon thermal cycling provided that the DNA-film is not subjected to a high voltage bias ($\pm$ 3V) at the highest temperature (80 °C). The most probable reason for the conductivity reduction is the creation of local defects (premelting), as indicated by Raman spectrum evolution (described below), analogous to that of local dislocations in a solid body during high temperature annealing. In the case of a solid body, local dislo-

cations can physically migrate at elevated temperatures and stop close to grain or phase boundaries [16]. Therefore local defects in DNA can be expected to also advance close to the tungsten nanoelectrodes, inducing irreversible changes in the molecule-metal contact characteristics [17]. We indeed observe such irreversibility in conductivity when the sample was maintained at 80 °C for 10-15 minutes with the maximum voltage (3V) of a chosen polarity. After cooling the sample back down to 30 °C, I(V) instability is observed on the positive branch (Figure 2c). When the sample was re-heated to 80 °C with V of the opposite sign, the instability appears on the negative branch of I(V) curve after cooling to 30°C. We observed such behavior up to 4 cycles of measurements.
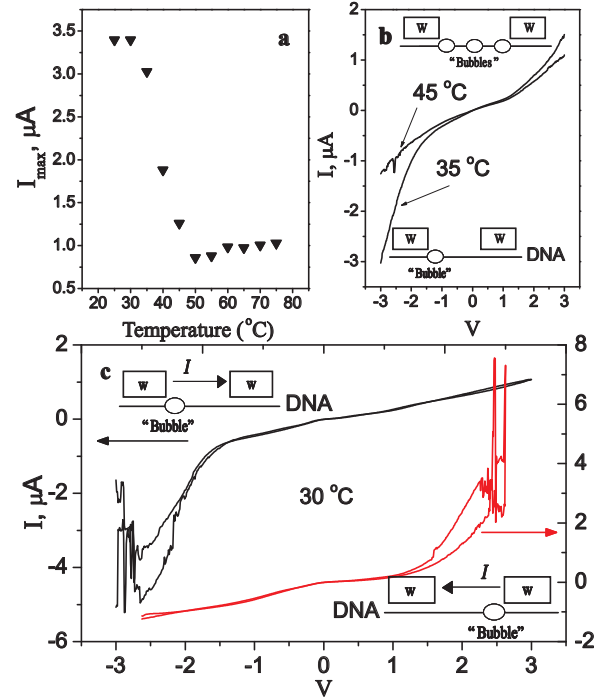

FIG. 2: Temperature dependent conductivity measurements under luminosity. a) Temperature dependence of I_{max}, b) $I-V$ characteristics (at 30 °C) of the DNA film at 35 and 45 °C. The insets show the increasing number of bubbles with a temperature, c) Irreversible $I-V$ characteristics of the DNA film which had been subjected to a high bias voltage at 80 °C with opposing polarities. The insets show the assumed schematics of the bubble type defect movements inside the DNA molecules at 80 °C.

In order to associate the conductivity changes with DNA structural modifications, we have tracked the structural evolution of DNA molecules by Raman spectroscopy. The 514.5 nm excitation line of an Ar$^+$-Kr$^+$ laser was focused on the samples through a ×50 magnification objective lense with a radiation power at source of 10 mW. The scattered light was analysed using a Jobin-Yvon triple grating spectrometer in the confocal configuration. The effective spectral resolution was less than

1 cm^{-1}. Raman spectra were taken in the 10-80°C temperature range on several films from the same batch as the conductivity measurements. The spectra of DNA-lipid complex taken at room temperature and at 80 °C as well as that of natural B-DNA (also taken in our lab) are compared in Figure 2. Temperature dependent Raman sepctra of the DNA-lipid complex films showed certain notable differences from those observed in DNA molecules in aquaous solution. First, a cooperative melting of double-stranded DNA was not observed even at 80°C, whereas the pre-melting effects in 10-65°C range were clearly present. We also remarked that nearly all vibrational modes exhibit a reversible temperature dependency during premelting after the heating and the subsequent cooling of the films (not shown). Structural rigidity of molecules imposed by the lipid intercalation may explain these differences. It should also be noted that the hypochromic effect [12] was not observed with exception of 1680cm^{-1} marker (see discussion below) due to the progressive change in the background luminescence of the film. Therefore, we have used the heating induced shifts in Raman peaks to track the premelting effects in DNA molecules. These peaks are sensitive to i) backbone and deoxynucleoside conformations, ii) interbase hydrogen bonding and iii) base stacking effect.

i) The broad peak at 780 cm^{-1} contains contributions from dC, dT and the 5'C-O-P-O-C3' backbone stretching. The peak frequency shifts down from 781 to 776 cm^{-1}) starting around 45°C until near 70°C (see Fig. 4a). The peak located at 840 cm^{-1} originates from the 5'C-O-P-O-C3' phosphodiester backbone movements and can be used as a quantitative measure of the ordered phosphodiester. This peak decreases in intensity and disappears into the background. The 746 cm^{-1} peak is the vibrational marker of C2'-endo/anti conformation of dT. This peak broadens and shifts strongly to lower frequency indicating the extended distribution of conformations at higher temperature (Fig. 4b). The onset of this movement is 55°C and continues to shift toward lower frequency up to 80°C.

ii) The bands at 1482 and 1573 cm^{-1} correspond to ring stretching vibrations of purine imidazole ring and are sensitive to hydrogen bonding. The both peaks shift to lower frequencies by 1 and 3 cm^{-1}, respectively, between 35 and 40°C and stabilize for temperature above 65°C (Fig. 4c). These shifts are the signature of the thermoinstability of base pairing. Moreover, the 3 cm^{-1} shift down of the Raman peaks between 1200-1400 cm-1 is associated with elimination of hydrogen bonding between bases (Fig. 4d).

iii) The intensity evolution of three peaks located at 1658, 1668 and 1682 cm^{-1} (inset of Fig. 4b) are normally attributed to the base stacking effects related to carbonyl stetching vibrations coupled to ring stretching vibration (mostly dT) [18]. The Raman peak at 1682cm^{-1} of the DNA-lipid film is; however, considerably more intense than in a typical B-DNA (see Fig 3), suggesting that contribution from the lipid complex cannot be ignored.
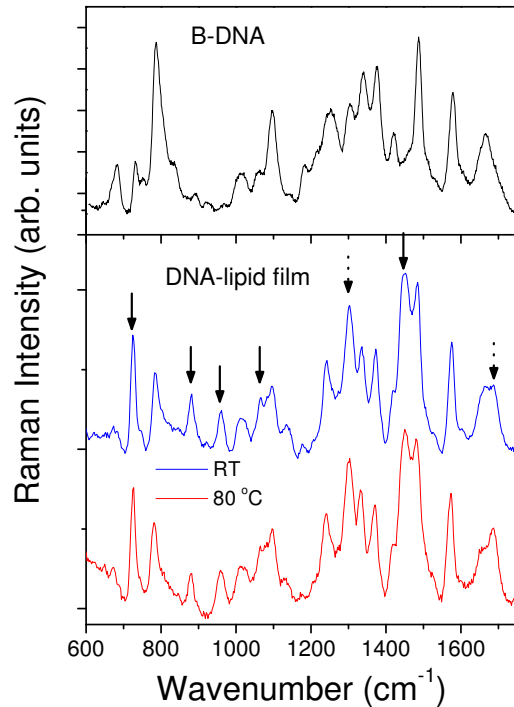

FIG. 3: Raman sepctra of natural B-DNA (top) and the DNA-lipid complex at room temerature and at 80°C. The solid arrows indicate the Raman peaks unique to the lipid complex. The dotted arrows indicated the peaks that contain contributions from both DNA molecules and the lipid complex. For detailed annotation of individual Raman bands, the readers are kindly asked to refer to literature, for example, [8],[15] and [18]. The Raman bands corresponding to the lipid-complex did not show heating induced shifting while certain DNA bands moved to lower wavenumbers. The DNA PO$_2^-$ symmetric stretching mode remains at 1092cm^{-1} through out the measurements indicating that the DNA molecules remains in their B-form without a signigicant change in the relative humidity around the molecues.

Hence we cannot conclude the hypochormicity observed here to the effect of DNA pre-melting without further investigation.

As described above, the premelting effects observed in DNA Raman isgnatures coincide with the temperature dependence of electrical conductivity measured in these films. Local destruction of double-stranded DNA conformation caused by premelting effects is most simply described as a "bubble" creation. The formation of such bubbles, and more importantly, its movement within the molecule is just like a dislocation loop moving in a metal microwire subjected to a high current density [19]. The insets in fig. 2c will help understand the observed changes in electrical conductivity (Fig. 2). Probably, in the presence of defects, the potential barrier in the vicinity of the

molecule-metal contact decreases. This barrier reduction is a known effect in metal-semiconductor microcontacts near a dislocation [20] and electromigration, the physical displacement of defects under an applied electrical current, is also well-known [21]. In electromigration, the direction of dislocation movement can be switched by changing the current direction. To estimate the pressure on a "bubble" by an electron wind, the current density in a DNA molecule must be known. Emerging consensus states that DNA molecules longer than 10 nm combined with bad electric contacts become insulating [22]. Therefore, only about 10 molecules at the film surface should be electrically active in our measurements. Taking 2 nm as the DNA diameter, the corresponding current density would be as high as 10^7 A/cm^2, comparable to a current density required to drag a dislocation in a metal microwire [19]. One can estimate the applied force on a bubble, F_b, using the equation for a dislocation in a metal [23]: $F_b = j m^* V_f S_b / e$, where j is the current density, m^* and V_f are the effective mass and Fermi velocity of the electrons injected in DNAs from the tungsten electrods

[24], S_b is the scattering cross section of the electrons by a bubble, and e is the electron charge. The applied force is about 1 pN (with $m^* \sim 10^{-30}$ kg and $V_f \sim 10^6$ m/s in W [25], $S_b \sim 1$nm^2 for a small bubble) which is enough to deform a DNA molecule [6]. The number of bubbles increases with a temperature [5], and it decreases the conductivity of DNAs (Fig. 2.b) and makes I-V characteristics more symmetrical (probably due to symmetrical molecule-metal contacts, see insets in Fig. 2b).

In summary, our measurements demonstrate that the electrical conduction in DNA can be compromised under a moderate heating above room temperature due to local disruptions in the long-range B-DNA structure. Furthermore, displacement of defects along molecules could explain why the structural transformation, as probed by Raman spectroscopy, is a reversible process while the electrical conductivity is not.

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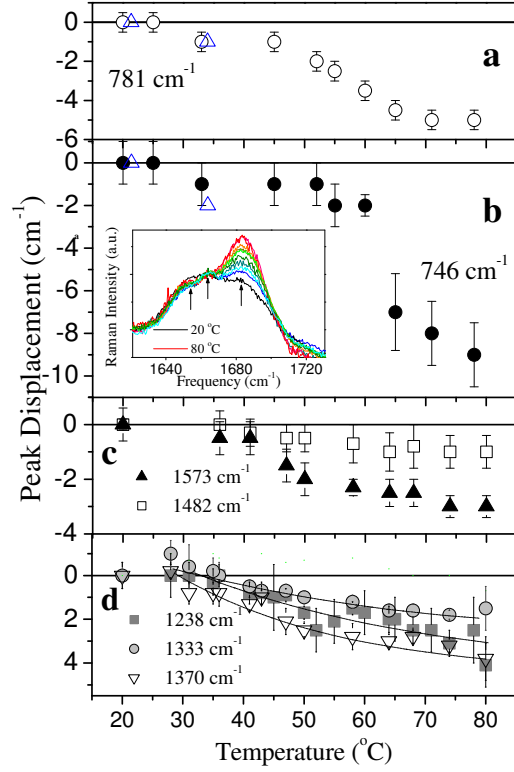[26] A. Yu. Kasumov *et al.*, Physical Review B, **72**, 033414 (2005)

FIG. 4: Temperature dependent pre-melting effect in Raman frequencies of **a:** The admixture of complex vibration of the B-DNA phosphodiester backbone conformation and thymine and cytosine ring modes [15], **b:** dT C2'-*endo* sugar pucker and *anti* glycosyl torsion mode [8] and **c:** dG and dA ring stretching vibrations sensitive to hydrogen bonding [15, 18] as well as dT [10]. The bands shift toward lower frequencies starting near 35 °C and the movement attenuates for 65 °C and higher for phosphodiester geometry and deoxyribose hydrogen bond vibrations. The onset of pre-melting effect at 746 cm^{-1} occurs near 50 °C. **d:** Interbase hydrogen bonding. The bands returns to their original position once the film is cooled back down to room temperature (see blue triangles in panels **a** and **b**). **inset b:** The intensity change near 1660-1680 cm^{-1} are observable from 35 °C and higher with no sign of attenuation. The spectra are normalized to the intensity values at 1664 cm^{-1} in order to show the relative intensity change among three bands (indicated by 3 arrows). The continued enhancement of 1680 cm^{-1} from room temperature to 80 °C is clearly observed. The bands at 1664 and 1650 cm^{-1} are indistinguishable at 20 °C (thick line) separate themselves into two distinct peaks at 1664 and 1648 cm^{-1} at 80 °C (thin line).